Characterization of genetic elements up-regulated in *Pseudomonas aeruginosa* PAO biofilms and transcriptional activity of the flagellar hook protein gene, *flgE*, during biofilm development

by

MARIA SUSANNA MEIRING

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DECLARATION

I declare that the dissertation, which I hereby submit for the degree Magister Scientiae at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university.

MARIA SUSANNA MEIRING

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DATE

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SUMMARY

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by

MARIA SUSANNA MEIRING

Supervisor: Dr J. Theron
Department of Microbiology and Plant Pathology
University of Pretoria

Co-supervisor: Prof. V.S. Brözel
Department of Microbiology and Plant Pathology
University of Pretoria

for the degree M.Sc

Pseudomonas aeruginosa, an opportunistic human pathogen, can grow in association with surfaces. Biofilm formation occurs in response to a variety of environmental signals and studies involving various different Gram-negative bacteria have shown that biofilms form in multiple steps, require intercellular signalling and demonstrate a profile of gene expression and cellular physiology that is distinct from that of planktonic cells. Although much progress has been made in the elucidation of genes and molecules necessary for bacterial attachment to surfaces and subsequent biofilm formation, much work is still needed to completely characterize the developmental process of biofilm formation. To investigate, previous attempts in our laboratory have relied on the isolation of genetic elements in *P. aeruginosa* PAO (DSM1707) that are up-regulated by attachment to a glass wool substratum. In this study, several of these attachment-induced genetic elements were genetically characterized and the transcriptional activity of one

of these regulatory elements was further investigated by reporter gene technology.

The nucleotide sequence of 24 attachment-induced genetic elements was determined and the results indicated that several of the genetic elements contained putative sigma 54 (σ^{54}) recognition sequences. Thus, σ^{54} may play an important role in biofilm formation as σ^{54} is known to regulate the expression of numerous genes under a wide range of different environmental conditions. One of the regulatory elements was selected and further characterized. Reporter gene technology, using the gene encoding the green fluorescent protein (GFP), was used to investigate the transcriptional activity of the regulatory element of the flagellar biosynthesis gene, *flgE*, in developing and well-developed *P. aeruginosa* biofilms under conditions of continuous flow using glass as substratum. *In vivo* detection of *flgE* expression in biofilms was performed by using fluorescent microscopy coupled with detailed inspection and comparison of images. The results indicated that transcription from the *flgE* promoter is up-regulated in specific single cells in the early stages of biofilm development as well as in cell clusters in 3-day old biofilms. Thus, flagella may not only play a role in the initial attachment of *P. aeruginosa* cells to the substratum, but also in the detachment of bacterial cells from the biofilm structures.

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LIST OF ABBREVIATIONS

A	absorbance
α	alpha
amp^r	ampicillin resistance
A/P ratio	ratio of β-galactosidase activity for the attached (A) cells to that of the planktonic (P) cells
ATP	adenosine triphosphate
β	beta
bp	base pair
ca.	approximately
°C	degrees Celsius
dATP	2'-deoxyadenosine-5'-triphoshate
dCTP	2'-deoxycitidine-5'-triphoshate
ddH_2O	deionized distilled water
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside-5'-triphosphate
DTT	dithiothreitol
e.g.	for example
Fig.	figure
GFP	green fluorescent protein
h	hour
IHF	integration host factor
IPTG	isopropyl β-D-thiogalactosidase
kb	kilobase pairs
lacZ	β-galactosidase gene
LB-medium	Luria-Bertani-medium
M	molar
mA	milliampere
MCS	multiple cloning site
mg	milligram

min	minute
ml	millilitre
mM	millimolar
NH_4OAc	ammonium acetate
OD	optical density
ONPG	2-nitrophenyl-β-D-galactopyranoside
ORF	open reading frame
PCR	polymerase chain reaction
PGW cells	planktonic cells grown in the presence of glass wool
RBS	ribosome binding site
RNA	ribonucleic acid
RPM	revolutions per minute
σ	sigma
SDS	sodium dodecyl sulphate
t	transcriptional terminator
T	translational terminator
TE	Tris-EDTA
tet^r	tetracycline resistance
TN-medium	tryptone-nitrate-medium
U	units
µg	microgram
µl	microlitre
UHQ	ultra-high quality
UV	ultraviolet
V	volts
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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